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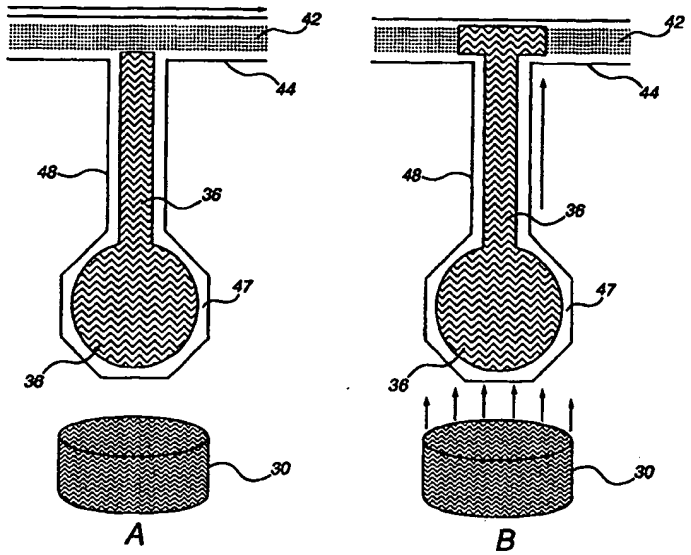

(57) Abstract

A new method is proposed for the precise manipulation of picoliter–nanoliter volumes in microfluidic chips. The technique relies on the thermal expansion of fluids whereby fluid pressure and flow is easily manipulated through control of the fluid temperature. Heat can be efficiently applied in a sample manner using a light/infrared source (30) (e.g., a halogen lamp) which selectively heats the fluid (36) in the chip device through absorption of the optical energy in the visible–infrared (VIS/IR) portion of the electromagnetic spectrum. Several applications for fluid control and manipulation on microfluidic chips are proposed using the VIS/IR–induced fluid pumping mechanism, including valving.

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THERMAL EXPANSION – INDUCED FLUID CONTROL FOR MICROFLUIDIC DEVICES

The present invention is an improvement of a commonly assigned patent application, Landers, J.P. Rapid Thermocycling For Sample Analysis, U.S. Serial No. 09/015,278, filed January 29, 1998, incorporated in its entirety by reference herein.

FIELD OF THE INVENTION

The present invention relates to methods and apparatus for performing precise manipulation of picoliter-nanoliter volumes in microfluidic chips. This technique specifically relies on thermal induced hydrodynamic pressure, wherein the thermal expansion of fluids controls the flow and pressure of fluids in microfluidic chip devices. Several applications for fluid control and manipulation on microfluidic chips are proposed using the thermal expansion-induced fluid pumping mechanism.

BACKGROUND OF THE INVENTION

The concept of microfabricated structures for total chemical analysis (microscale total analysis systems-μTAS) embodies a design wherein the total analysis of complex samples is carried out in a completely integrated "chip". The μTAS or "Laboratory-on-a-Chip" concept involves miniaturization of benchtop analytical procedures into microfluidic chips roughly the size of a double-wide microscope slide (~ 100 mm. x 100 mm x 0.5 mm). The improvement in functionality is driven by the basic concept that an overall reduction in the dimensions of fluid volume, handling and flow should result in the enhancement of analytical performance. Harrison, D.J.; Flurri, K.; Seiler, K.; Fan, Z.; Effenhauser, C.S.; Manz, A. Micromachining a Miniaturized Capillary Electrophoresis-Based Chemical Analysis System on a Chip. Science 1993, 261, 895. The chip architecture is composed of microfluidic channels and other three-dimensional structures on a suitable substrate (glass, plastics, etc.) with microchannel dimensions in the micrometer range and reservoir dimensions in the millimeter range.

The compact design and production of these devices allow for some standard liquid separation methods, like capillary electrophoresis, to be miniaturized into a format that is roughly 10-times smaller in size than conventional systems and allows for separations to be carried out roughly an order of magnitude faster. For example, U.S. Patent No. 5,770,029 discloses an electrophoretic microdevice for use in a variety of electrophoretic applications, including clinical assays. Despite the fact that rapid, microscalar analyses are possible with microfluidic chips, the potential of this platform lies in integrating other chemistries and sample manipulation processes into the very same chip used for electrophoretic analysis.

Sample treatment steps, such as cleanup procedures, concentration steps, and derivatization protocols preceding or following the actual separation step are now being developed and incorporated into microfluidic chips. Cheng, J.; Shoffner, M.A.; Hvichia, G.E.; Kricka, L.J.; Wilding, K. Chip PCR. 11. Investigation of different PCR amplification systems in microfabricated silicon-glass chips. Nucleic Acids Res. 1996, 24, 380. Since the primary component on the chip is a microfluidic channel that allows for the electrophoretic separation of the sample components, multiple separation paths with identical geometry can be constructed on a single chip and used for massive parallel processing of samples. In addition to the high-throughput capabilities of such a system, the miniaturization of a total analysis system in the form of an integrated chip would make the analysis of samples faster, more accurate, and, most important, less costly for almost all areas of chemical analysis, including environmental sample analysis and clinical diagnostics.

A prerequisite for the development of a functional microfluidic chip is the ability to precisely manipulate liquids and control their movement through the complex architecture of the microfluidic system. Currently, the manipulation of liquids and transport of analytes in microchip devices is realized using two main principles: i) electrokinetic phenomena, e.g., electrophoretic and electroosmotic effects and ii) hydrostatic pressure.

Fluid flow via electroosmotic flow has been described extensively in the capillary electrophoresis literature. See Chang, H.T. and Yeung, E.S., "Self-regulating Dynamic Control of Electroosmotic Flow in Capillary Electrophoresis," Anal. Chem. 65,650(1993). Electroosomotic flow relies on the surface charge of the channel, the ionic strength of the solution and the strength of an electric potential applied across the solution. For example, U.S. Patent number 5,755,942 describes a system for processing a plurality of reactions comprising a sample channel for moving samples into a microlaboratory array wherein the control of samples having features that diffract light at various wavelengths are optically monitored by a device that can electrokinetically control the flow of the sample. U. S. Patent No. 4,908,112 discloses a silicon semiconductor wafer for analyzing biological samples by directing the motion of liquids through the wafer by electroosmosis.

Elaborate electrokinetic injection schemes on microfluidic chips have been developed for either sample injection in a continuously proportional mode or injection with a fixed plug length using switching potentials between reservoirs. Seiler, K.;Fan, Z.H.; Flurri, K.; Harrison, D.J. Electroosmotic Pumping and Valveless Control of Fluid Flow within a Manifold of Capillaries on a Glass Chip. Anal. Chem. 1994, 66, 3485. The combination of the compact, virtually dead volume-free design of the microchip devices and the electrokinetic control of picoliter volumes with high precision are the hallmark of the highly reproducible manipulation of fluids in microfluidic devices. However, this technique is limited to solutions whose chemical composition allow for electroosmotic flow, e.g., low ionic strength solutions. Many solutions, such as chaotropic agents or biological fluids where the ionic strength is high, suppress the electroosmotic effect and, therefore, may not provide adequate electroosmotic flow and/or cannot be controlled precisely through voltage switching. It is known that the mobility of large DNA fragments (> 25kbp) is zero under a constant electric field due to constant reorientation of the molecule. Also, cells and particulates that do not exhibit uniform surface charges or are entirely devoid of surface charges, cannot be manipulated easily by an electrical field. Thus, under these conditions, the methods currently available for manipulation of liquids and transport of analytes in microchip devices are ineffective. The diverse nature of the analytes to be analyzed on electrophoretic chips make it essential that a method independent of electroosmotic flow be devised so that a diverse range of solutions can be manipulated in a controlled, reproducible manner. Only then will the precise control of small flow rates (picoliter -nanoliters per minute) be possible so that complex fluids like blood, urine and saliva can be accurately manipulated, and integrated chemistries, like sample preparation (including solid phase extraction), be executed.

Fluid flow based on hydrostatic pressure is also utilized in capillary electrophoresis (CE) for sample injection and for rinsing the capillary between electrophoretic separations. However, it is frequently assumed that the pressure driven pumping of fluids suitable for microfluidic devices is easily controlled by mechanical means, e.g., through valves. This is, in fact, uncertain and problems may be encountered if hydrodynamic pressures (5-80 psi) similar to pressures in CE are applied to an electrophoretic chip.

Variations in the pressure of a central pressurized gas/air supply causes problems in miniaturized systems not encountered in the "macroworld" of capillary electrophoresis. A reliable and practical interface for the plumbing from an external pressure source to the microfluidic chip constitutes a considerable challenge that is not easily solved. Therefore, there is a need for a hydrodynamically-driven fluid pumping technique for microfluidic devices.

U.S. Patent No. 5,375,979 discloses a method for creating hydrodynamic pressure on a microstructure, including a thermal micropump having a working chamber, intake valve and

discharge valve. The pump action of the micropump is achieved through a succession of overpressure and partial-vacuum cycles through a succession of heating and cooling steps of gas. This micropump has a disadvantage of being quite complex, and presents valves tending to experience high leakage rates, and is of questionable functionality.

Burns, M.A., Mastrangelo, C.H., Sammarco, T.S., Man, F.P., Webster, J.R. Johnson, B.N., Forester, B., Joones, D., Fields, Y., Kaiser, A.R., Burke, D.T., Proc. Natl. Acad. Sci. USA, 93, 5556-5561 (1996), describe the movement of liquid by heating the interface of a liquid plug in a microchannel at the meniscus. This method, however, requires the incorporation of heating elements along the channel to move the liquid drop inside the channel. This complicates the design of the chip and is less flexible. In addition the introduction of air in microchannel systems is unfavorable due to the high surface tension of air which is usually only overcome by high pressure heads in the microchannel.

U.S. Patent No. 5,639,423 (Northrup, et al) discloses the use of Lamb-wave pumps to effect fluid flow in a microfluidic device. Such pumps, however, are very difficult to control, given the transducers used are operational only at a narrow band of alternating voltage frequencies corresponding with the resonant frequency of the transducer. Furthermore, fluid control is not continuous over a wide range of flow rates. Lamb-wave pumps also are difficult to fabricate, occupy a relatively large amount of area on the chip, and require mechanical components, which are prone to wear/fatigue.

Accordingly, an advance in the art would be achieved with a method and apparatus for the generation and precise control of hydrodynamic pressure for fluid manipulation in microfluidic devices without the use of an external pressure source or the need to rely on electroosmotic flow or Lamb-wave pumps.

SUMMARY OF THE INVENTION

The present invention meets the need for an effective hydrodynamic fluid flow based source that does not require an external pressure source or electroosmotic flow, providing methods and apparatus for accurate control of fluid flow in microfluidic devices. The invention allows the provision of methods and apparatus comprising microfabricated structures for microscale total analysis systems.

This invention discloses a method for precisely manipulating liquids and controlling their movement through microfluidic systems in a manner that is not only noncontact, but also independent of solution ionic strength and surface charge. This method, referred to herein as thermal expansion-induced fluid pumping, precisely controls movement of small volumes (pico- to nanoliter range) in microfluidic networks by relying on the thermal expansion of

fluids as the driving force for fluid flow. As used herein, the term "VIS/IR-induced fluid pumping" means the pumping of fluids, in a microfluidic system, through use of a heat source not in contact with the system, which heat source, when directed toward the fluid of interest, causes that fluid to expand through thermal expansion, the heat source including light energy in the visible and infra-red range.

As used herein, the term "thermal expansion-induced fluid pumping" includes VIS/IR-induced fluid pumping, and further includes other mechanisms for heating fluids on a microfluidic chip to achieve thermal expansion of the fluid, including other wavelengths of light, e.g., UV, and other heating methods such as providing thin heating films on the chip, and heating the fluid with heated forced air.

As used herein, the term "microfluidic chip" is intended to include, but is not necessarily limited to, devices which have been microfabricated to contain one or more structural elements, such as channels, reaction chambers, etc., with minimal dimensions ranging from tens of microns (the dimensions of biological cells) to nanometers (the dimensions of some biological macromolecules), i.e., "mesoscale," and even smaller. The term "mesoscale" is used herein to define chambers and flow passages having a cross sectional dimension on the order of 0.1 μm to 500 μm. The term "microfluidic chip" is further intended to include, but not necessarily be limited to, microfabricated devices which perform microscale and/or mesoscale chemical reactions including precise control of parameters of the reaction; the parameters controlled by such devices may include, e.g., temperature, pressure, concentration of reactants, the intensity or frequency of incident light, electromagnetic fields, ultrasonic pressure waves, etc.

It is preferred within the invention to have a small, sealable reservoir filled with the expandable liquid that is heated from ambient temperature to a higher temperature. The liquid expands and applies pressure to the walls of the reservoir and subsequently exits through its only outlet, a microchannel at the base of the reservoir, creating a constant flow of fluid through the channel.

It is preferred within the invention that the heat source be provided remotely, for example, by a halogen lamp that specifically heats the fluid through absorption of VIS/IR optical energy. The rate and extent of fluid expansion is a function of the rate and the extent of temperature change, which is easily controlled by monitoring the temperature of the liquid.

It is a further object within the invention to create a number of microchannel/reservoir geometries that would allow VIS/IR-induced fluid pumping to govern flow on microchips in a manner that is independent of electroosmotic flow or an external pressure source.

A further object within the invention is to use an organic liquid with a high thermal expansion coefficient and that is immiscible with aqueous solutions. For example, toluene has a thermal expansion coefficient that is approximately 5-times larger than an aqueous solution.

The internal pressure (at constant volume) generated due to the expansion of the heated organic liquid can be estimated from thermodynamics to be several atmospheres with several degrees change in temperature in the reservoir. This expansion can be effectively transferred to the aqueous solution in order to achieve higher flow rates of that aqueous solution that would be impossible by heating the aqueous solution alone.

It is a further object of this invention to control the flow of aqueous solutions in microfluidic devices by using an organic liquid that can act as a chemical valve, by virtue of its immissibility with the aqueous solution, blocking the aqueous solution from flowing through microfluidic channels.

Other objects and advantages of the present invention will become apparent from the following description and the accompanying drawings. Generally, this invention discloses a system that can precisely control the flow of small volumes in microfluidic devices using thermal expansion-induced fluid pumping. The system of using thermal expansion-induced fluid pumping can be utilized in chemical reaction systems for synthesis or processing of organic, inorganic, or biochemical reactions and separations.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is an illustration of the microchannel configuration for a microchip designed to use thermal-induced fluid pumping according to one embodiment of the present invention.

Figure 2 is an illustration of a microfluidic pump designed to use thermal-induced fluid pumping according to one embodiment of the present invention.

Figure 3 is an illustration of a microfluidic valve designed to use thermal-induced fluid pumping according to one embodiment of the present invention.

Figure 4 is an illustration of a thermoreservoir connected to a plurality of liquid reservoirs designed to use thermal-induced fluid pumping according to one embodiment of the present invention.

Figure 5 is an example of how the thermoreservoir in concert with valves designed to use VIS/IR induced fluid pumping can continuously pump fluid in a microfluidic device.

Figure 6A illustrates temperature versus time in ten thermocycles achieved with heating according to the present invention.

Figure 6B illustrates temperature versus time as measured in the heating/cooling block of a conventional thermocycling instrument.

Electrophoretic "chips" consist of glass, plastic, silicon, or gallimarsenide substrates into which a pattern of microchannels, often simple but sometimes complicated, have been fabricated. Embodiments of such chips are shown in Figure 1. The microchannels terminate at reservoirs which are often drilled holes in the structure itself and which hold volumes as low as a few microliters to as much as several hundred microliters. These reservoirs may also be prepared using known techniques, such as photolithography and etching. Other microfabrication technologies include, but are not limited to sputtering, electrodeposition, and low-pressure vapor deposition. The use of crystalline materials, such as silicon or gallium arsenide as substrates for microfabricated devices offer certain advantages over non-crystalline materials such as glass. Crystalline semiconductor materials provide etched surfaces comprising crystal planes, the shapes and dimensions of which can be precisely controlled. Further, crystalline materials may be bonded by processes such as fusion at elevated temperatures, or with a field assisted method such as Mallory bonding. Semiconductors offer the further advantage of permitting electronic circuiting to be integrated with the microfabricated device using conventional IC fabrication techniques.

Microchannel structures on electrophoretic chips are typically in the micrometer range, with depths ranging from 10-50 μm and widths from 30-100 μm. A microchannel with the dimensions of 10 μm (deep) x 50 μm (wide) x 3 mm long has a total microchannel volume of 1.5 nanoliters (nL). Therefore, to flow the equivalent of 20 microchannel volumes of a given solution through this particular microchannel would require the pumping of 30 nL of solution. The controlled flow of these ultralow volumes through the microchannels of microfabricated electrophoretic devices may be difficult to regulate with hydrostatic pressure. The low volume pumping of solution on chips is more conducive to EOF which can be controlled quite accurately by the magnitude of the voltage applied. A limitation associated with EO-driven flow, however, is its dependence on two parameters: 1) the ionic strength of the constituents of the solution being pumped; and 2) the chemical composition of the microchannel wall. For example, a low, negligible EOF is likely to result when using solutions containing a large salt concentration, such as 100 mM NaCl or 2X Tris-borate-EDTA (TBE) solutions. This fact precludes use of EOF for most biological reactions, which must be executed in biological buffers, as is the case with polymerase chain reactions. Isotonic solutions are high in ionic strength and are, therefore, incompatible with EOF.

Low EOF will also result when the interior channel walls are composed of a neutral or nonionic substance such as plastic. Under such conditions, EO flow is likely to be of limited use with respect to its ability to pump solution through the microchannel architecture. This limits the use of microfabricated electrophoretic devices as the basic element in the

"laboratory on a chip" concept where a diverse array of biological and chemical solutions may need to be effectively pumped at the same time through the microchannel architecture.

The present invention overcomes these limitations in the art. The ability to do accurate temperature cycling on localized volumes of solution ranging from as low as a few microliters to up to several hundred microliters, such as through the methods and apparatus taught herein, can be exploited for accurate pumping of small volumes (nL-μL) of solution through microchannel structures. The basis for the pumping is thermal expansion of liquids. In a confined space, a change of 100°C can result, for example, in the generation of up to 10 psi within the chamber; if the only exit from the chamber is a microchannel, fluid can be forced from the chamber through the microchannel. The flow rate can be controlled very accurately by changes in temperature as little as 1-2°C. The relative accuracy of the pumping, e.g., control of pressure and flow rate, is a function of the accuracy of the temperature control, e.g., precisely raising the temperature of the fluid from a first temperature to a second temperature in a precisely controlled time-frame. The absolute accuracy of the pumping operation, e.g., how accurately the quantity of fluid being pumped is controlled is a function of the temperature control, channel geometry and liquid properties (e.g., homogeneous versus non-homogeneous). As will now be apparent to one of ordinary skill in the art, the microdevice can be specifically designed to meet the desired pumping requirements.

The present invention is a microfluidic device that employs a thermal expansion-induced fluid pumping mechanism. The system of employing thermal expansion-induced fluid pumping in microfluidic devices provides precise fluid control, that is more effective than the methods of controlling fluid flow in microfluidic devices as described in the prior art. This system can be used in an array for a high-throughput microreaction unit. Applications of the thermal expansion-induced microfluidic method are numerous and can generally encompass any analytical system that can be employed at a nano- to pico-liter scale. Multiple reagents can be utilized in a reaction on such a device where they are contained in reservoirs that are interconnected to one another on the microfluidic device, and the addition of each reagent into a reaction chamber can be precisely controlled in order to successfully produce the desired product. The microfluidic device can be used to chromatographically separate samples, spectrophotometrically detect the samples as well as collect the separated samples. This invention essentially discloses a concept applicable to developing microfabricated structures for total chemical analysis on a microscale.

Fluid flow according to the present invention is achieved by heating fluid contained in a sealed reservoir, causing the fluid to expand through one or more microchannels. This fluid flow can be reversed by cooling the fluid remaining in the reservoir, which causes the fluid to contract. The heating and cooling of fluid contained in the fluid reservoir is accomplished remotely, i.e., using heating/cooling sources that do not directly contact the

microfluidic chip or the reservoir. In the case of heating, the reservoir is remotely heated preferably using a radiation source, the wavelength of which matches the absorbance properties of the fluid in question, the intensity and focus of which can be precisely controlled to effect the desired amount of heating of the fluid in the reservoir. Using select optical energy sources allows for heating primarily the fluid itself, rather than the container or microfluidic chip and other structural components. This, in turn, allows for rapid heating, and therefore rapid and precise transition from an initial, lower temperature to a higher temperature associated with expansion and flow of the fluid. IR-mediated heating is primarily achieved through the absorbance of radiation by molecules in the fluid sample, for example, water molecules in the sample being pumped. Of course, other heating sources to induce thermal expansion and flow of the fluid of interest can also be used, and include, for example, heating coils, lasers, and heated forced air. See also U.S. Patent No. 5,589,136 (Northrup), which discloses the use of doped polysilicon for heating a reaction chamber and bulk silicon for convection cooling. Further, any heat or light source, the energy from which can be manipulated by optical filters and/or lenses to effect absorbance of such energy by the fluid of interest in order to heat the fluid can be used.

A suitable reservoir according to the methods of the present invention is one in which extremely low volumes of sample can be effectively heated and cooled, including sample volumes in the nanoliter range. The reservoir must be made of a material that allows the penetration of the desired wavelengths of light, such materials including by way of example but not limitation quartz glass, glass, silicon, transparent plastics, and the like. Preferably, the reservoir or container will have a high surface-to-volume ratio. A high surface-to-volume ratio leads to a decrease in the thermal time constant, which can lead to an increase in the efficiency of heating and cooling cycles. A high surface-to-volume ratio, while not as important for the heating step, is related to the effectiveness of the cooling step. Various examples of suitable reservoirs, include but are not limited to, microchambers, capillary tubes, and microchips.

A preferred example of a suitable reservoir is a microchamber made from thin-walled glass. Another preferred embodiment is a glass capillary tube. Such capillaries are typically used in capillary electrophoresis ("CE"). Suitable inner diameters of the capillaries having an outer diameter of about 370 μm typically vary between about 15 μm and 150 μm. Thermal gradients that lead to convection are substantially reduced in capillary tubes. Glass capillary tubes are commercially available from Polymicron Technologies, Phoenix, AZ.

Another preferred example of a suitable reservoir is the channel structure incorporated into a microfabricated device, such as the microfabricated substrate described by Wilding and co-workers in *Nucleic Acids Res.*, 24:380-385 (1996).

Any other reservoir can be used according to the methods of the present invention, provided that the vessel is made of a material which allows IR or other radiation to directly heat the sample. Further guidance in preparing such microfabricated devices is provided, for example, in U.S. Patent Nos. 5,250,263; 5,296,114; Harrison et al., *Science* 261:895-897 (1993); and McCormick et al., *Anal. Chem.*, 69:2626-2630 (1997), incorporated by reference herein.

Heating of the fluid is preferably accomplished through the use of optical energy from a noncontact heat source. While contact heating sources can also be used in accordance with the present invention, they are less preferred, as they are not as quickly or precisely able to effect fluid heating and allow for cooling as the non-contact sources.

In general, the wavelength of the radiation source must match the absorbance properties of the fluid being heated in order to maximize heating effects on the fluid by the radiation. Wavelengths in the near infrared region are preferred, given that nearly everything shows absorbance in that region. Preferably, this optical energy is derived from an IR light source which emits light in the wavelengths known to heat water, which is typically in the wavelength range from about 0.775 μm to 7000 μm. For example, the infrared activity absorption bands of seawater are 1.6, 2.1, 3.0, 4.7 and 6. 9 μm with an absolute maximum for the absorption coefficient for water at around 3 μm. Of course, if the fluid being heated is organic, the optical energy should be of a wavelength that matches the absorbance properties of the organic liquid. In some cases, it may be desirable to use multiple energy sources and multiple wavelengths in order to match absorbance properties of different fluids being heated on the chip. Furthermore, different wavelengths can be employed to heat the same fluid at different rates in different regions of the chip, in order to provide different flow rates. The IR wavelengths are directed to the vessel containing the sample, and because the vessel is made of a clear or translucent material, the IR waves act directly upon the sample to cause heating of the sample. Although some heating of the sample might be the result of the reservoir itself absorbing the irradiation of the IR light, (particularly with silicon substrates) heating of the sample is primarily caused by the direct action of the IR wavelengths on the sample itself.

It is important to note that while the use of IR and near-IR wavelengths will be preferred for many applications, in certain instances visible and even UV wavelengths may be used, depending on the nature of the fluid and its absorbance characteristics.

Heating and fluid expansion and flow can be effected in either one step, or numerous steps, depending on the desired application. For example, a particular methodology might require that the sample be heated to a first temperature, allowing a first fluid expansion and flow to occur, maintained at that temperature for a given dwell time, then heated to a higher temperature, allowing a second fluid expansion and flow to occur, and so on. As many

heating steps as necessary can be included. Similarly, continuous heating, whereby the temperature of the fluid is continuously and uniformly raised, may be used to provide continuous fluid flow over finite temperature ranges for limited heating cycle times.

Similarly, cooling to a desired temperature can be effected in one step, or in stepwise reductions with a suitable dwell time at each temperature step, or over a continuous cooling step for limited times and temperature ranges. Positive cooling is preferably effected by use of a non-contact air source that forces air at or across the reservoir. Preferably, this air source is a compressed air source, although other sources could also be used. It will be understood by those skilled in the art that positive cooling results in a more rapid cooling than simply allowing the vessel to cool to the desired temperature by heat dissipation. Cooling can be accelerated by contacting the reservoir with a heat sink comprising a larger surface than the reservoir itself; the heat sink is cooled through the non-contact cooling source. The cooling effect can also be more rapid if the gas from the non-contact cooling source is at a lower temperature than ambient temperature. This can be accomplished in any number of ways, including using chilled air, nitrogen, etc., or forced air directed at the chip. Cooling can also be accomplished, of course, by removing the energy (heat) source or changing its wavelength to one of lower absorbance for the fluid being heated.

An apparatus capable of achieving the desired heating and cooling in order to effect thermal expansion/contraction of fluids in microfluidic chips when employed according to the teachings herein is shown and described in co-pending U.S. Patent Application Serial No. 09/015,278, incorporated in its entirety by reference herein.

Figure 6A shows a plot of temperature versus time (seconds) in ten thermocycles run according to the present invention, in which two temperature stages are achieved. The amount of time that elapses between the highest temperature is very small, with the temperature change being effected in less than 1 second. Similarly, the change from the lowest temperature back to the highest temperature takes generally less than one second, and can be done as quickly as 300 to 400 milliseconds. This rapid temperature change translates into fast response times for initiating and halting pumping and valving operations using the present-invention. In contrast, Figure 6B is representative of a typical temperature profile for one three-temperature thermocycle obtained from a commercially available thermocycling instrument. The dotted line represents the temperature of the heating/cooling block of the apparatus, and the solid line the temperature of the sample. It can be seen that the time lags between the temperatures – the highest to lowest temperature about 30 seconds, the lowest to intermediate temperature about 15 seconds, and the intermediate to highest temperature about 25 seconds – is sufficiently longer than that achieved by the methods and apparatus of the present invention. Such long time lags would render the prior art device impractical for pumping or valving fluids in microfluidic chip applications. Desired temperature changes in

the sample itself take even longer to achieve. The temperature transitions in the sample are not nearly as sharp as those obtained in the block, or those in the sample according to the present methods. More specifically, the temperature transition of the sample from the highest to lowest temperature takes about 100 seconds, and from the intermediate to highest temperature about 80 seconds. This time lag becomes important in certain processes where rapid changes in fluid flow are desired, for example, when using the present invention as a chemical valve. Referring to Fig. 6A, the lower temperature points may be viewed as an open valve scenario, which, according to the present invention, may be quickly closed by heating the valving fluid to the higher temperature.

The non-contact means for directly heating the sample can be any means known in the art for generating the desired range of wavelengths in the near IR wavelengths. Typically, the heating means will be an IR source, such as an IR lamp, an IR diode laser or an IR laser. An IR lamp is preferred, as it is inexpensive and easy to use. Preferred IR lamps are halogen lamps and tungsten filament lamps. Halogen and tungsten filament lamps are powerful, and can heat several samples flowing in parallel. A tungsten lamp has the advantages of being simple to use and inexpensive, and almost instantaneously (90% lumen efficiency in 100 msec) reach very high temperatures. Such lamps are commercially available from General Electric, Cleveland, OH. A particularly preferred lamp is the CXR, 8V, 50W tungsten lamp available from General Electric. This lamp is inexpensive and convenient to use, because it typically has all the optics necessary to focus the IR radiation onto the sample; no expensive lens system/optics will typically be required.

The IR source is positioned remotely (non-contact) from the sample, such that light emanating from the lamp impinges the sample. In one embodiment, the lamp is positioned remotely to the actual sample, and the radiation from the lamp is transferred via optically IR-transmissible fiberglass or through a combination of lenses and mirrors. When using optical fibers the ends of the fibers are mounted in a platform such that they are close to, (e.g., a few millimeters or less) but do not touch, the chip. The chip is slid into position on the platform, which maintains consistent orientation from chip to chip relative to the IR source. Other embodiments in which the sample is placed in the path of the IR light source are equally within the scope of the present invention.

Although it may not be necessary, the heat source can be manipulated, such as through the use of lenses and filters mounted between the lamp and the reaction vessel. Such lenses and filters serve to focus the radiation as well as to eliminate wavelengths that could interfere with the fluid expansion taking place and/or the temperature sensing. Filtering and focusing of the IR light using, for example, IR transmissible lenses or filters is preferred, as this reduces the occurrence of temperature gradients in the sample or partial boiling of the sample.

As used herein, the term "direct" or "directly" when used in reference to heating means that the sample itself is heated by the heating source, such as through absorption of IR radiation, as opposed to heating of the vessel containing the sample which in turn heats the sample. Direct heating of the sample is, therefore, achieved even when lenses, filters and the like are used.

As used herein, the term "non-contact" with respect to an energy source, means that the energy source is directed at, or focused on, a region of the microfluidic chip desired to be heated, and may be quite close to that region, but does not physically touch it.

As used herein, the term "remote" with respect to an energy source, includes non-contact sources, but also includes truly remote sources of energy, which are not themselves near or focused at the microfluidic chip, but are brought to bear on the microfluidic chip by some intermediary, such as optical fibers, which transmit the energy from the remote heat source to the region of interest on the microfluidic chip.

The apparatus of the present invention uses finely tuned temperature ramping, which allows for rapid heating cycles of the sample. In addition, heat transfer problems frequently encountered with solid block heaters are eliminated with the non-contact approach of the present invention. The heater can be powered by any means, such as a low voltage power supply. Preferred is a 5 volt DC supply system, although it will be understood that any other suitable means can also be employed.

Cooling can be achieved by any non-contact means known in the art for positively cooling an object. Accordingly, the non-contact cooling source should also be positioned remotely to the sample or reaction vessel, while being close enough to effect the desired level of heat dissipation. Both the heating and cooling sources should be positioned so as to cover the largest possible surface area on the sample vessel. The heating and cooling sources can be alternatively activated to control the temperature of the sample. It will be understood that more than one cooling source can be used.

Positive cooling of the reservoir dissipates heat more rapidly than the use of ambient air. The cooling means can be used alone or in conjunction with a heat sink. A particularly preferred cooling source is a compressed air source. Compressed air is directed at the reservoir when cooling of the sample is desired through use, for example, of a solenoid valve which regulates the flow of compressed air at or across the sample. The pressure of the air leaving the compressed air source can have a pressure of anywhere between 10 and 60 psi, for example. Higher or lower pressures could also be used. The temperature of the air can be adjusted to achieve the optimum performance in the cooling process. Although in most cases compressed air at ambient temperature can create enough of a cooling effect, the use of cooled, compressed air to more quickly cool the sample, or to cool the sample below ambient temperature might be desired in some applications.

A means for monitoring the temperature of the sample, and a means for controlling the heating and cooling of the sample, are preferably also provided. Generally, such monitoring and controlling is accomplished by use of a microprocessor or computer programmed to monitor temperature and regulate or change temperature. An example of such a program is the Labview program, available from National Instruments, Austin, Texas. Feedback from a temperature sensing device, such as a thermocouple, is sent to the computer. Other temperature sensing devices, such as resistive heaters (RTD's or Resistive Temperature Devices), can also be used. Such heaters may be bonded to the chip, and used to measure temperature by measuring electrical resistance through the heater. See generally Burns, et al., Microfabricated Structures for Integrated DNA Analysis, Proc. Nat. Acad. Sci., USA Vol. 93 pp. 5556-5561 (1996). In one embodiment, the temperature sensing device provides an electrical input signal to the computer or other controller, which signal corresponds to the temperature of the sample. The rate of heating/cooling of the fluid in the reservoir can be used to determine the rate of fluid expansion/contraction and hence the flow rate of fluid from the reservoir into the microchannels. Preferably, the temperature sensor, which can be coated or uncoated, can be placed in a temperature sensing reservoir placed adjacent to the fluid reservoir containing the sample to be pumped. The temperature sensing reservoir should be of the same type as the sample fluid reservoir, only containing a blank, such as water or a buffer solution instead of sample. Alternatively, the thermocouple can be placed directly into the sample fluid reservoir, provided that the thermocouple does not interfere with or affect the heating/cooling of the fluid, and provided that the thermocouple used does not act as a heat sink. A suitable thermocouple for use with the present invention is constantan-copper thermocouple. In a highly preferred embodiment, microfabrication sensors (p/n-junctions) can be used as the temperature sensor. In some instances it might be advantageous to sense the sample temperature through a thermosensor directly measuring the reaction vessel, or the sample itself. Other temperature sensing methods that comprise non-contact temperature sensing devices are: optical pyrometers, primary acoustic thermometers, or spectroscopic techniques for measuring temperature of liquids.

Signals from the computer, in turn, control and regulate the heating and cooling means, such as through one or more switches and/or valves. The desired temperature profile, including dwell times, is programmed into the computer, which is operatively associated with heating and cooling means so as to control heating and cooling of the sample based upon feedback from the thermocouple and the predetermined temperature profile.

In a preferred embodiment, the optical energy is focused on the sample by means of IR transmissible lenses so that the sample is homogeneously irradiated. This technique avoids "hotspots" that could otherwise result in the creation of undesirable temperature differences and/or gradients, or the partial boiling of the sample. The homogeneous treatment

of the sample vessel with optical energy therefore contributes to a sharper temperature profile and more uniform and reproducible fluid expansion. The homogenous sample irradiation can further be enhanced through the use of a mirror placed on the opposite site of the IR source, such that the reservoir is placed between the IR source and the mirror. This arrangement reflects the radiation back onto the sample and substantially reduces thermal gradients in the sample. Alternatively, the radiation can be delivered by optical IR-transparent fiberglass, for example, optical fiberglass made from waterfree quartz glass (Fasertyp IR-QQ, Schott Glawerke Weisbaden, Germany) that is positioned around the reservoir and that provides optimal irradiation of the sample.

Referring now to FIG 1, there is illustrated an example of one possible embodiment of the invention, in this case a microfluidic device designed to perform microscale total chemical analysis on a microfluidic device. It will be appreciated that this is an illustrative example only of a preferred application of the invention, and a wide array of other uses of the invention is possible. The sample to be analyzed is placed in a sample loading reservoir 4 in the microfluidic device. The sample loading reservoir is preferably then sealed, with the exception of an opening leading to a microchannel 6. Sealing of the reservoir may be accomplished by filling the reservoir, then sealing it using known methods during production of the chip. Alternatively, the reservoir may be sealed with one or more valves, whereby the valve acts to seal the reservoir when the valve is closed, and upon being opened, acts to allow the reservoir to be refilled. Such valving can be accomplished using known methods, such as "virtual" valving, which relies on low Reynolds number flow of fluids through intersecting channels which are used to divert flow by controlling the pressure in the channels. See Brody, et. al, "Biotechnology at Low Reynolds Numbers," Biophysical Journal, Vol. 71, pp. 3430-3441 (1996). Other valving techniques, such as use of chemical valves of the present invention, discussed in greater detail hereinafter, may also be used. Using thermal expansion-induced pumping, the sample is heated and undergoes expansion, causing the sample to flow from the loading reservoir 4 through a first microchannel 6 until it reaches a purification chamber 8. The purification chamber 8 may be used, for example, in situations where the sample is a complex mixture of diverse macromolecules and the investigator needs to purify the molecule that he or she wants to study in order to successfully conduct the experiment of interest. From the purification chamber 8 the sample flows in to a second microchannel 10. The second microchannel 10 is intersected at intersection 11 by a third microchannel 12 that is connected to a reaction reagent reservoir 14. This intersection 11 may be used as a "virtual" valve, previously described, or as a chemical valve, as will subsequently be described. Also using VIS/IR induced pumping, reagent from the reaction reagent reservoir 14 can be pumped for mixing with the sample. Such mixing can occur in the microchannel

10, and/or in the reaction vessel 16, depending on the timing of pumping the reagent from the reagent vessel 14.

Following the completion of a predetermined reaction in the reaction chamber 16, the sample flows into a sample injection microchannel 18. The sample injection microchannel 18 is intersected at intersection 19 by a separation channel 22. The flow of the sample into and through the separation channel 22 is controlled by reservoirs 20 which, in conjunction with the intersection 19, act as valves, and will be subsequently described. From the separation channel 22, the sample flows into a detection device 24 where it is analyzed, for example, spectrophotometrically. From the detection device 24, the sample flows through a sample collection microchannel 26 and then to a sample collection reservoir 28.

It will now be appreciated that each of the reservoirs 4, 14 and 20 can be heated and/or cooled according to the present invention in order to induce fluid flow from or to those reservoirs. Similarly, while VIS/IR induced pumping can be used to effect fluid flow at every point of the process, it need not be used exclusively, rather, may be employed in conjunction with other fluid flow techniques such as those employed in the prior art.

FIG 2 illustrates one type of VIS/IR induced pumping according the present invention. A sealed reservoir 34 contains an organic liquid 36 with high thermal expansion properties and an aqueous liquid 38. The organic liquid 36 is immiscible with the aqueous liquid 38. The sealed reservoir 34 is connected to a microchannel 40. The sealed reservoir 34 is exposed to a heating / cooling source 30 whereby the flow 32 of heating irradiation or cooling air contacts the sealed reservoir 34 whereby, in the case of heating, the organic liquid 36 expands, forcing the aqueous liquid 38 to flow out of the sealed reservoir 34 into the microchannel 40. In the case of cooling, the organic liquid 36 contracts, causing the aqueous liquid 38 to flow back into and/or in the direction of, the sealed reservoir 34.

FIGS. 3A and 3B illustrate a VIS/IR induced chemical valve according to the teachings of the present invention. An aqueous liquid-containing microchannel 44 intersects an organic liquid-containing microchannel 48 that is connected to an organic liquid-containing reservoir 47. The flow of an aqueous liquid 42 flowing through the aqueous liquid microchannel 44 can be stopped by remotely heating the organic liquid 36 with the heating source 30, causing it to expand out of the organic liquid microchannel 48 into the aqueous liquid microchannel 44. Since the aqueous liquid 42 is immiscible with the organic liquid 36, the flow of the aqueous liquid 42 is stopped, as illustrated in FIG 3B. Upon cooling of the organic liquid 36, the organic liquid 36 contracts, causing it to flow out of the aqueous liquid microchannel 44 and back into the organic liquid microchannel 48. This causes the flow of the aqueous liquid 42 to flow unimpeded in the aqueous liquid microchannel 44, as in FIG 3A. Preferably, the walls of the aqueous liquid-containing microchannel 44 either comprise, or are coated with, a hydrophilic material, and the walls of the organic liquid-containing

microchannel 48 either comprise, or are coated with, a hydrophobic material, to further enhance the valving capabilities of the invention. It will now be appreciated that, by alternately heating and cooling the organic liquid 36, using the remote heating/cooling sources of the present invention, that a reliable on/off valve can be conveniently and effectively achieved and used repeatedly in a microfluidic flow control environment. It will also now be appreciated that chemical valves of the present invention can be advantageously employed in a number of practical applications, such as hydrostatic injection.

FIG 4 illustrates how the mixing of reagents on the microfluidic device can be achieved according to the teachings of the present invention. A thermoreservoir 58 containing an aqueous liquid 38 is connected to several reagent reservoirs 52 by means of wide-bore microchannels 53. Reaction reagents, 46, 48 and 50, are contained in reagent reservoirs 52 in a lyophilized form. When the thermoreservoir 58 is heated by a heating/cooling source 30, the aqueous liquid 38 thermally expands into the reaction reagents reservoirs 52, whereby the lyophilized reagents, 46,48 and 50 are dissolved. When the thermoreservoir cools, the aqueous liquid 38 containing the dissolved reaction reagents, 46, 48 and 50 contracts back into the thermoreservoir 38 from the reagent reservoirs 52 whereby the reaction reagents, 46,48 and 50 are mixed. Further mixing can be accomplished by repeating the procedure any number of times. The mixture can flow into the remaining microfluidic device architecture by means of a narrow-bore microchannel 54 that is connected to the thermoreservoir 58.

FIG 5 shows a thermal expansion driven micro-pump that can continuously achieve pumping action according to the teachings of the present invention. A sealed thermoreservoir 63 is connected to a liquid reservoir 64, which need not be sealed, by means of a microchannel 54 that is intersected by a valve 66. The thermoreservoir 63 is also connected to the remaining microfluidic device architecture by means of microchannel 67 that is also intersected by a valve 69. The valves 66 and 69 may, but need not be, VIS/IR valves as previously discussed in reference to FIGS. 3A and 3B. When the thermoreservoir 63 is heated by the heating/cooling source 30, the valve 69 is open while the valve 66 is closed, causing the aqueous solution 38 to flow in the direction 72 from the thermoreservoir 63 through the microchannel 67 to the remaining microfluidic device architecture. When the thermoreservoir 63 is cooled, the valve 69 is closed, while valve 66 is open, causing the aqueous solution 38 in the liquid reservoir 64 to flow in the direction 74 into the thermoreservoir 63.

It will be readily appreciated that the reservoir 64 can be made larger relative to the thermoreservoir 63, allowing for a virtually limitless supply of fluid 38 over the duration of the microfluidic operation.

It will now be understood that the above descriptions are made by way of illustration, and that the invention claimed herein may take other forms within the spirit of the methods

and structures described herein. Variations and modifications will occur to those of ordinary skill in the art, and all such variations and modifications are considered to be part of the invention, as defined by the following claims, including all equivalents thereof.

CLAIMS

We claim:

1. A method of inducing fluid flow in a microfluidic chip containing a sealed reservoir holding a first fluid, said chip further including a first microfluidic channel in communication with said reservoir, comprising the step of remotely heating the first fluid in said sealed reservoir, thereby causing said first fluid to thermally expand and flow from said reservoir through said first microfluidic channel.

2. The method of claim 1, wherein said step of remotely heating is achieved using a non-contact visible/infrared light source.

3. The method of claim 1, including the step of monitoring the temperature of said fluid and controlling the rate of heating thereof in order to obtain a desired flow rate of said first fluid.

4. The method of claim 1, wherein said first microfluidic channel intersects a second microfluidic channel, through which a second fluid flows, whereby the flow of said first fluid acts to impede the flow of said second fluid, as said first fluid flows to the intersection of said first and second microfluidic channels.

5. The method of claim 4, wherein said first and second fluids are immiscible.

6. The method of claim 5, including the additional step of cooling said first fluid, thereby causing said first fluid to withdraw from said intersection, thereby allowing said second fluid to resume flowing through said second microfluidic channel.

7. The method of claim 4, wherein one of said first and second fluids is hydrophobic, and the microchannel through which it flows is provided with a hydrophobic surface, and the other of said first and second fluids is hydrophilic, and the microchannel through which it flows is provided with a hydrophilic surface.

8. The method of claim 1, wherein said sealed reservoir contains a second fluid, said second fluid being immiscible with said first fluid, whereby said second fluid is remotely heated,

and thereby expanded, transferring the resulting fluid expansion to said first fluid, which flows through said first microfluidic channel.

9. The method of claim 1, wherein said step of remotely heating is achieved using an energy source the wavelength of which corresponds to a wavelength absorbed by the first fluid.

10. The method of claim 8, wherein said first fluid is an aqueous solution, and said second fluid is an organic liquid with a higher thermal expansion coefficient relative to the first liquid.

11. The method of claim 1, further including the step of cooling the fluid in said reservoir in order to reverse the flow of said first fluid in said microfluidic channel.

12. The method of claim 11, wherein said cooling step is achieved using chilled compressed air.

13. The method of claim 1, wherein said first fluid flows to a mixing reservoir, said mixing reservoir containing a component to be mixed or dissolved with said first fluid.

14. The method of claim 13, wherein said first fluid is withdrawn from said mixing reservoir by remotely cooling said first fluid in said sealed reservoir, and additional mixing is achieved by remotely heating said fluid in said sealed reservoir and causing said first fluid to flow back into said mixing reservoir.

15. The method of claim 1, wherein said chip further includes at least one additional fluid, said first fluid and said additional fluid exhibiting different absorbance properties, said method including the step of irradiating each said fluid with energy having a wavelength corresponding to that at which each said fluid absorbs said energy.

16. The method of claim 1, wherein said first fluid is heated by at least two different wavelengths of energy.

17. The method of claim 1, wherein said first fluid is remotely heated using light passed through at least one optical fiber.

18. The method of claim 10, wherein said first fluid is remotely heated with an energy source having a first wavelength, and said second fluid is heated with an energy source having a second wavelength.

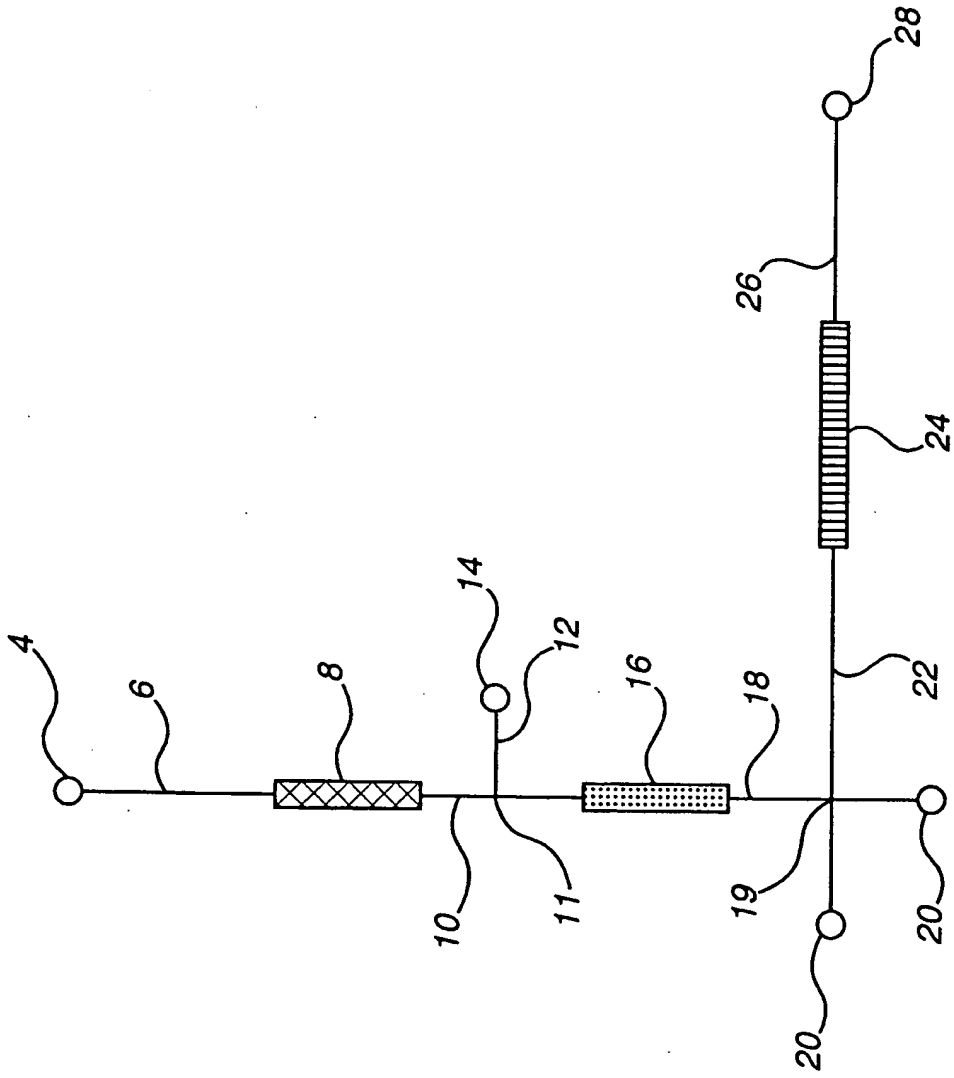

FIGURE 1

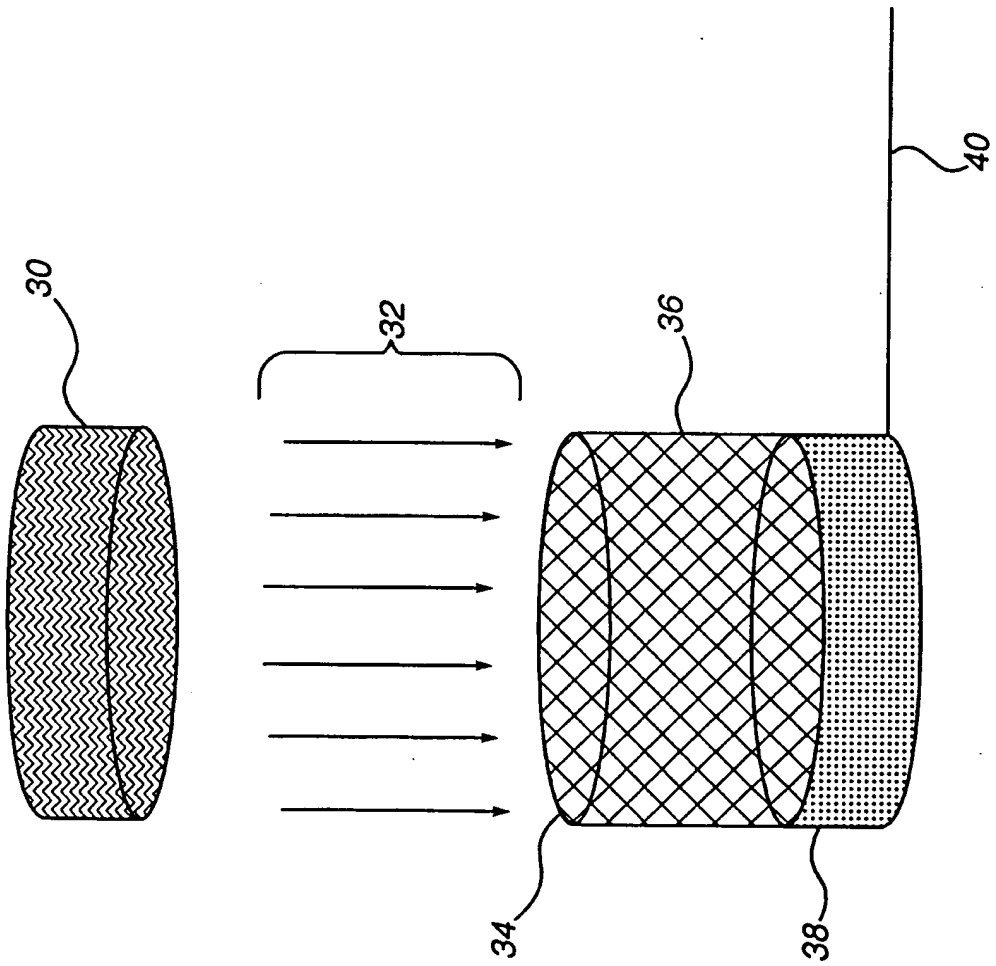

FIGURE 2

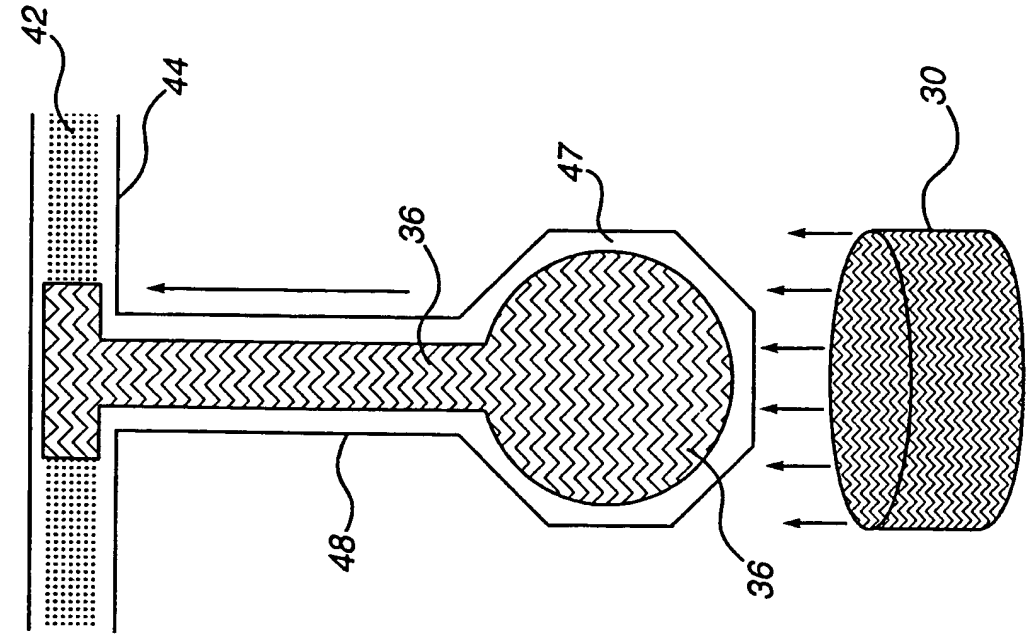

FIGURE 3B

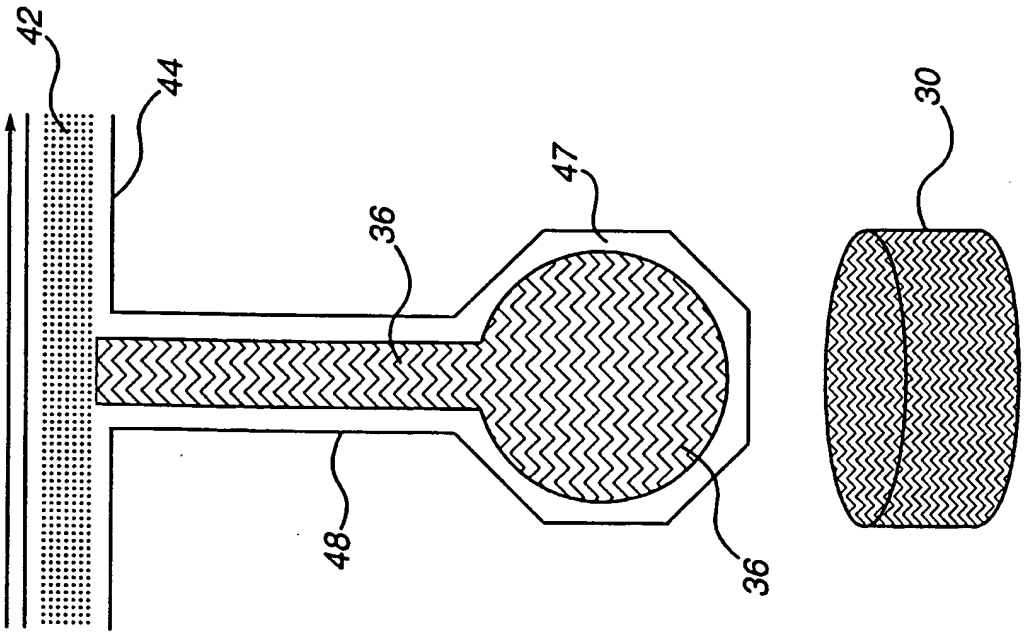

FIGURE 3A

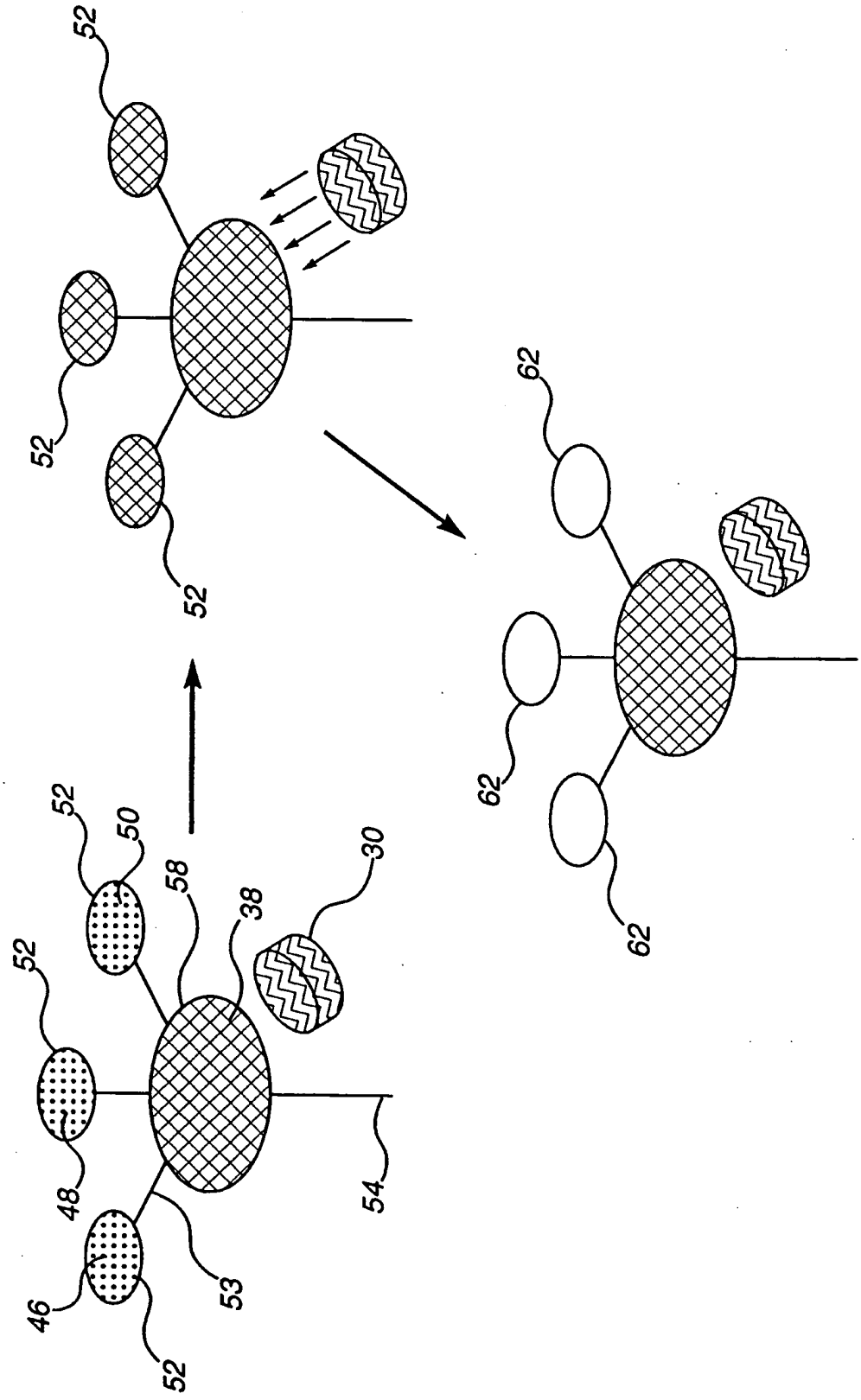

FIGURE 4

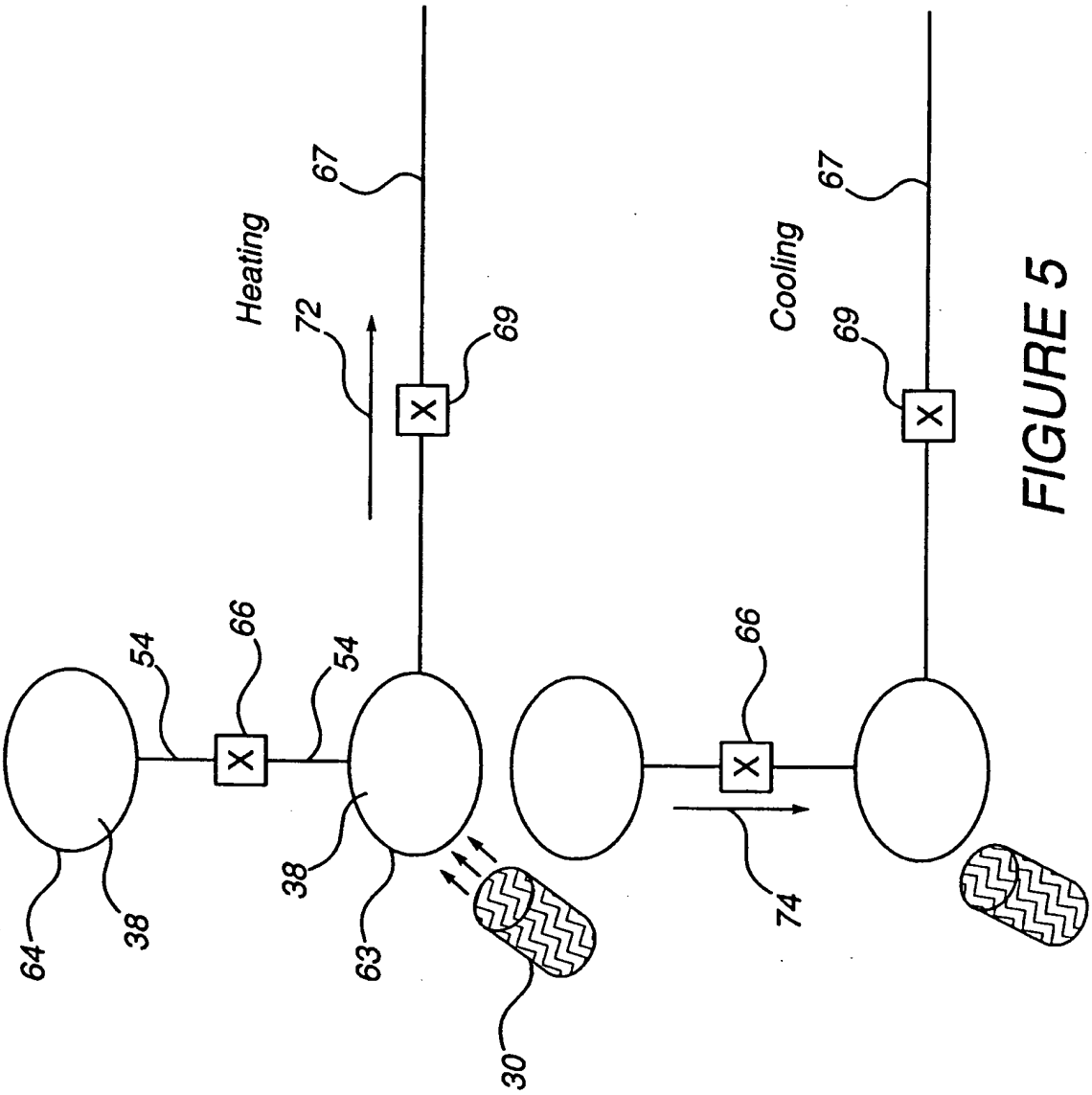

FIGURE 5

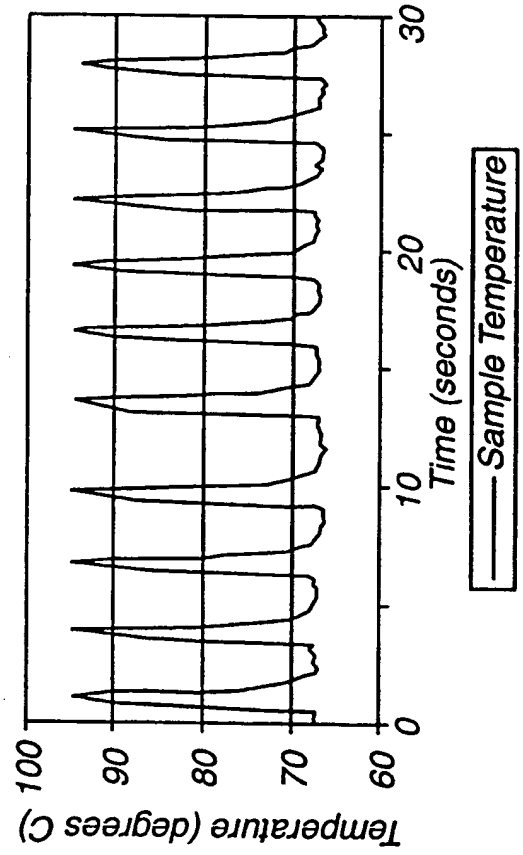

FIGURE 6A

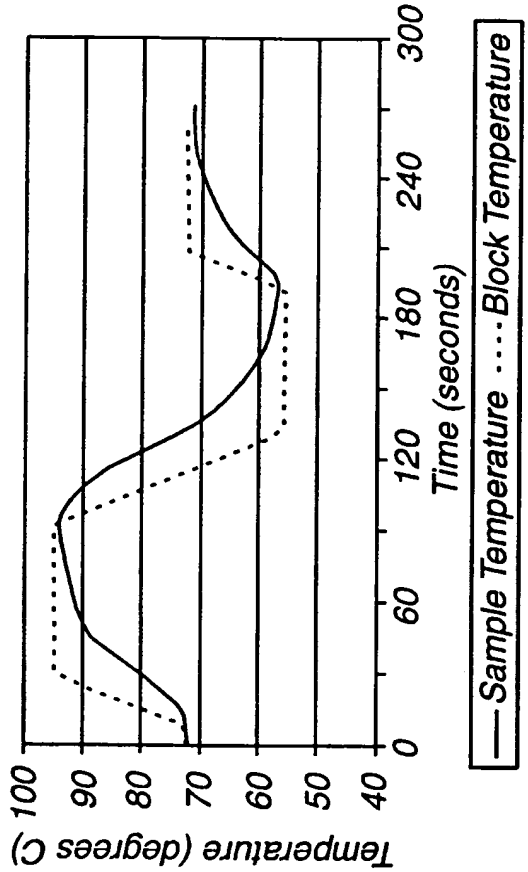

FIGURE 6B

INTERNATIONAL SEARCH REPORT

Inte .onal Application No
PCT/US 99/01831

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 F16K13/10 B01L3/00 C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 F16K B01L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 700 695 A (LINGANE PAUL J ET AL) 23 December 1997	1
Y	see column 2, line 45 - line 61	4-7,9,10
X	see column 10, line 14 - line 57; figures 10,12	8,11,13, 14
X	see column 8, line 54 - column 9, line 17	2,3

Y	US 4 676 274 A (BROWN JAMES F) 30 June 1987	4-7
	see column 1, line 24 - line 30	
	see column 3, line 45 - line 56	
	see column 8, line 11 - line 57; figures 8-10,12,15-18	

Y	EP 0 568 024 A (CANON KK) 3 November 1993	9
	see column 8, line 9 - line 28; figure 7	
A	see column 11, line 25 - line 35; figure 16	17

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
18 May 1999	28/05/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Hocquet, A

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Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Inter onal Application No
PCT/US 99/01831

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 97 02357 A (AFFYMETRIX INC ;ANDERSON ROLFE C (US); LIPSHUTZ ROBERT J (US); RAV) 23 January 1997 see page 51, line 31 - page 52, line 15	10
A	see page 66, line 15 - line 32	4-7

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inte .onal Application No
PCT/US 99/01831

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